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Lab Resource: Stem Cell Line

Derivation of the human embryonic stem cell line RCe012-A (RC-8)

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ABSTRACT

The human embryonic stem cell line RCe012-A (RC-8) was derived from a frozen and thawed day 5 embryo cultivated to the blastocyst stage. The embryo was voluntarily donated as unsuitable and surplus to fertility requirements following ethics committee approved informed consent under licence from the UK Human Fertilisation and Embryology Authority. The cell line shows normal pluripotency marker expression and differentiation to the three germ layers in vitro. It has a normal 46XX female karyotype and microsatellite PCR identity, HLA and blood group typing data is available.

Resource table

Name of stem cell construct	RCe012-A
Alternative name	RC-8, RC8
Institution	Roslin Cells Ltd.
Person who created resource	B. J. Tye, K. Bruce, P. Dand, G. Russell, D.M. Collins, A. Greenshields, H. Bradburn
Contact person and email	Paul.desousa@roslincells.com; Paul.desousa@ed.ac.uk Janet.downie@roslincells.com Aidan.courtney@roslincells.com Malcolm.bateman@roslinfoundation.com
Date archived/stock date	06 September 2010 (seed bank)
Type of resource	Biological reagent: cell line
Sub-type	hESC, research grade
Origin	Blastocyst with ICM and trophoblast
Key transcription factors	Oct4 (confirmed by flow cytometry)
Authentication	See Quality Control test summary, Table 1
Link to related literature (direct URL links and full references)	N/A
Information in public databases	http://hpscreg.eu/cell-line/RCe012-A
Ethics	Informed consent obtained. Scotland A Research Ethics committee approval obtained (07/MRE00/56). Conducted under the UK Human Fertilisation and Embryology Authority licence no R0136 to centre 0202.

Resource details

RCe012-A (RC-8) was derived from a thawed day 5 blastocyst that was surplus to requirement or unsuitable for clinical use. The cell line was derived by whole embryo outgrowth on mitotically inactivated human fibroblast (HDF) feeder cells using xeno-free medium (XF KODMEM) and expanded under xeno free and feeder free conditions.

By flow cytometry, RCe012-A (RC-8) expressed the pluripotency makers Oct-4, Tra-1-60 and SSEA-4 (94.7%, 89.4% and 99.8%, respectively), whereas low expression of the differentiation marker SSEA-1 (2.2%) was observed (Fig. 1, Table 1). Differentiation to the three germ layers, endoderm, ectoderm and mesoderm, was demonstrated using embryoid body formation and expression of the germ layer markers α-fetoprotein, β-tubulin and muscle actin (Fig. 2).

A microsatellite PCR profile has been obtained for the cell line, and HLA Class I and II typing is available (Table 2). Blood group genotyping gave the blood group AO_1 (Table 2).

Verification and authentication

The cell line was analysed for genome stability by G-banding (Fig. 3) and showed a normal 46XX female genotype. The cell line is free from mycoplasma contamination as determined by RC-qPCR. Microsatellite PCR DNA profiling for cell identity is available (Table 2).

Materials and methods

Ethics

Derivation of hESC from surplus to requirement and failed to fertilise/develop embryos was approved by The Scotland A Research Ethics Committee and local ethics board at participating fertility clinics and conducted under licence no R0136 from the UK HFEA with informed donor consent.

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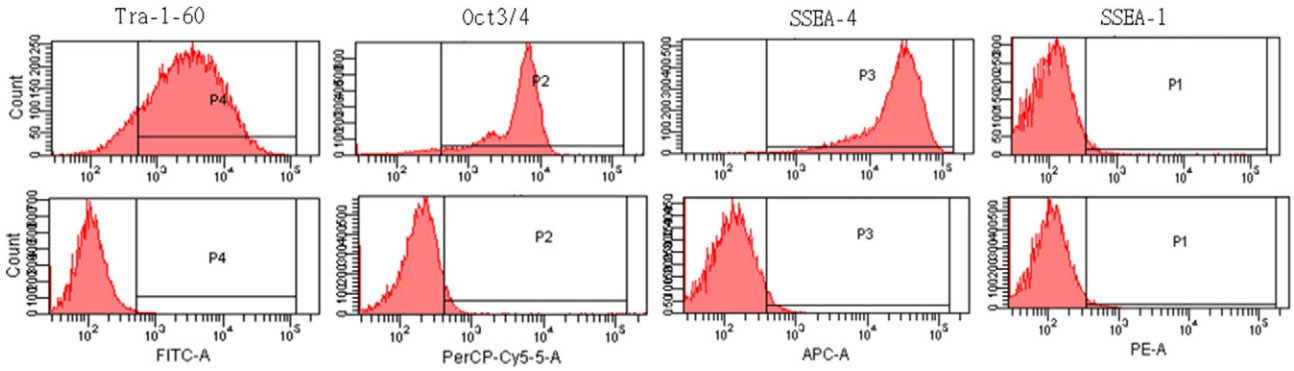

Fig. 1. RCe012-A (RC-8) was subjected to flow cytometry analysis for markers of pluripotency with specific antibody (top row) or isotype control (bottom row) as indicated above the histograms. Percentage staining is indicated in Table 1.

Cell culture

Vitrified embryos were thawed using Vitrified Embryo Safety Thawing Pack (Kitazato/Dibimed, Valencia, Spain) according to manufacturers' instruction and were cultured in system SAGE Quinn's Advanced Blastocyst medium (Rochford Medical, Coventry) after day 3 of development. Embryos were cultured at 36.5–37.5 °C, 5 ± 0.5% CO_2, 5 ± 0.5% O_2 in drops under paraffin oil (Rochford Medical) and transferred to fresh medium at least every 2–3 days.

By day 8 of development, embryos were placed in derivation conditions consisting of mitotically inactivated neonatal human dermal fibroblasts (HDFs) (ThermoFisher Scientific (Cascade Biologics), Paisley, UK) on tissue culture plastic in XF KODMEM medium (Knockout-DMEM, 15% KOSR-XF, 2 mM L-glutamine, 1% MEM Non-essential amino acids, 2% XF Growth Factor Cocktail, 0.1 mM β-mercaptoethanol, all ThermoFisher Scientific, Paisley, UK) supplemented with 80 ng/ml human bFGF (ThermoFisher Scientific). Assisted hatching was performed by removing the zona pellucidae mechanically using Swemed Cutting tools (Vitrolife, Göteborg, Sweden).

HDF cells were cultured in DMEM (Lonza, Slough, UK), 10% FCS (GE Healthcare (PAA), Buckinghamshire, UK) and 2 mM L-glutamine (ThermoFisher Scientific). HDFs were mitotically inactivated using gamma irradiation at 50GY using a Gammacell Elite 1000 machine. For use as a feeder layer, irradiated HDFs were plated at 50,000 cells/cm^2 in XF KODMEM medium supplemented with 80 ng/ml human bFGF (ThermoFisher Scientific). Cells were cultured at 36.5–37.5 °C, 5 ± 0.5% CO_2, 5 ± 0.5% O_2 and 50% medium exchanged 6 days a week.

The established cell line was expanded and banked using CellStart matrix and Stempro hESC Serum Free Medium (ThermoFisher Scientific). Passaging was performed mechanically using an EZ passage tool (ThermoFisher Scientific). hESC lines were expanded to 25–30 wells of a 6-well plate and cryopreserved in 0.5-1 ml Cryostor CS10 (Biolife Solution, Washington, USA).

Mycoplasma

Mycoplasma detection was performed using Applied Biosystems PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit and MicroSEQ™ Mycoplasma Real-Time PCR Detection Kit (ThermoFisher Scientific (Applied Biosystems)) according to manufacturer's instruction.

Endotoxin

Endotoxin levels were determined using the Kinetic-QCL assay (Lonza) and an incubating plate reader (BioTek ELx808) according to manufacturer's instructions. Briefly, an unknown sample was compared with a standard curve of known levels of control endotoxin. An assay was deemed valid if the coefficient of correlation, r ≥ 0.980 and the CV (%) for the standard curve was ≤10%.

Table 1
Summary of quality control testing and results for RCe012-A (RC-8).

Classification	Test	Purpose	Result
Donor screening	HIV 1 + 2 Hepatitis B Hepatitis C	Donor screening for adventitious agents	Negative
Identity	Microsatellite PCR (mPCR)	DNA profiling to give cell line its signature, gender/species	Performed
Phenotype	Flow cytometry	Assess antigen levels & cell surface markers commonly associated with hESC	Oct 3/4: 94.7% Tra 1-60: 89.4% SSEA-4: 99.8% SSEA-1: 2.2%
Genotype (details provided in Table 2)	Blood group genotyping (DNA analysis) Karyology (G-banding) HLA tissue typing	To establish blood group of the line Confirmation of normal ploidy by G-banding To establish full HLA type I and II genotype of the line	O1O1 46XX HLA typed class I and class II
Microbiology and virology	Mycoplasma Endotoxin	Mycoplasma testing by RT-qPCR Screening for endotoxin levels	Negative 0.54 EU/ml
Morphology	Photography	To capture a visual record of the line	Normal
Differentiation potential	Embryoid body formation	To show differentiation to three germ layers	Expression of muscle actin, β-tubulin and α-feto protein

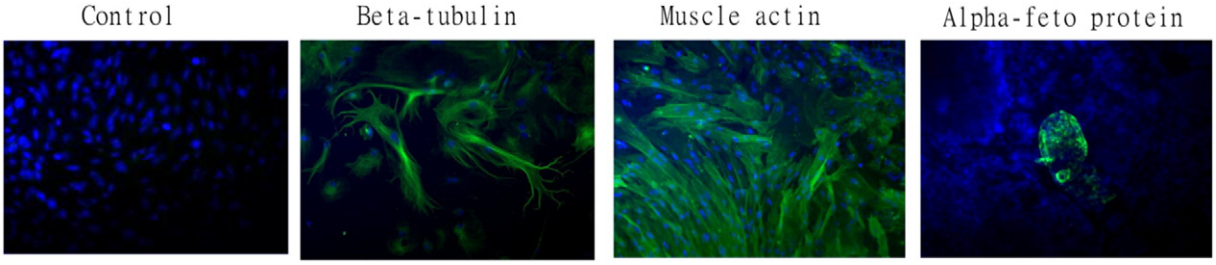

Fig. 2. In vitro differentiation of RCe012-A (RC-8) to ectoderm (β-tubulin III), mesoderm (muscle actin), and endoderm (α-fetoprotein). Specific staining shown in green, cell nuclei are counterstained with DAPI (blue).

Flow cytometry

Pluripotency was determined using the Human and Mouse Pluripotent Stem Cell Analysis kit (BD, Oxford, UK). Oct 3/4 and SSEA-4 were included as pluripotency markers, and SSEA-1 as a differentiation marker. FITC conjugated Tra-1-60 (BD) was used as an additional pluripotency marker. Fixed and permeabilised cells were analysed using a FACS Aria flow cytometer (BD). Percentage expression of each marker was compared to isotype control or unstained cells.

Immunocytochemistry

hESC were fixed in methanol (ThermoFisher Scientific), blocked using 10% goat serum (Sigma-Aldrich, Dorset, UK) in PBS (Lonza) containing 0.01% Tween-20 (Sigma) and stained with AFP (1:500; Sigma), β-tubulin III (1:1000; Sigma), muscle-specific actin (1:50; DAKO, Glostrup, Denmark), and secondary antibody anti-goat IgG-AlexaFluor 488 (1:200; ThermoFisher Scientific). Images were acquired using a Zeiss S100 Axiovert fluorescence microscope or Nikon eC1 confocal microscope.

In vitro differentiation

Confluent hESC cells lifted using a cell scraper (Corning) and embryoid bodies EBs generated in ultra low attachment plates (Corning) in EB medium (20% FBS (GE Healthcare (PAA)), 80% KO-DMEM 1 mM L-glutamine, 0.1 mM β-mercaptoethanol, 1% nonessential amino acids (all ThermoFisher Scientific)). After 9 days in suspension culture, EBs were being transferred onto glass slide tissue culture chambers (Nunc, ThermoFisher Scientific) coated with 0.1% gelatin (Sigma) at 0.1 ml/cm^2 and cultured for 14 days.

Genomic analysis

All outsourced assays were carried out under a Quality and Technical Agreement. DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Manchester, UK) according to manufacturer's recommendations and provided in recommended quantities to the service providers.

Microsatellite PCR, or Short Tandem Repeat analysis, was used to determine cell line identity and was carried out by Public Health England. A profile was obtained for the following core alleles: vWA, D16S539, Amelogenin, THO1, CSF1PO, D5S818, D75820, D135317 and TPOX.

Human Leukocyte Antigen (HLA) tissue typing was carried out by the Scottish National Blood Transfusion Service.

Blood group genotyping was carried out by the Molecular Diagnostics laboratory at NHSBT.

Karyotype analysis was carried out by The Doctors Laboratory (London, UK) or the Western General Cytogenetics Laboratory (Edinburgh, UK) Live cells at 60–70% confluency were shipped overnight in warm containers, fixed and analysed by standard G-banding analysis. For research grade lines, 20 spreads were analysed.

Acknowledgements

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Images of embroid body staining were kindly provided by S. Greenhough and J. Gardner, Roslin Cellab Ltd.

Table 2
Microsatellite PCR, blood group and HLA tissue typing results for RCe012-A (RC-8).

Microsatellite PCR results							
D3S1358 1	D3S1358 2	vWA 1	vWA 2	D16S539 1	D16S539 2	D2S1338 1	D2S1338 2
14	16	17	19	12	13	17	20
Amelogenin 1	Amelogenin 2	D8S1179 1	D8S1179 2	D21S11 1	D21S11 2	D18S51 1	D18S51 2
X	X	14	16	28	30	12	16
D19S433 1	D19S433 2	THO1 1	THO1 2	FGA 1	FGA 2	CSF1PO 1	CSF1PO 2
14	14	7	9.3	23	26	12	13
D5S818 1	D5S818 2	D7S820 1	D7S820 2	D13S317 1	D13S317 2	TPOX 1	TPOX 2
9	11	8	10	11	12	8	8
Blood group genotyping							
RhD	RhC	Rhc	RhE	Rhe	Fy a	Fy b	Fy GATA
pos	pos	neg	neg	pos	pos	pos	neg
Jka	Jkb	K	k	M	N	S	S
neg	pos	neg	pos	pos	pos	pos	pos
Kp a	Kp b	Do a	Do b	ABO			
neg	pos	pos	pos	O1O1			
HLA tissue typing							
HLA class I type		HLA-A*02, A*32; B*15, B*38; C*03, C*12					
HLA class II type		HLA-DRB1*04, DRB1*15; DRB4*01; DRB5*01; DQB1*06, DQB1*03					
Comment		C*03 is expressed serologically as C10, B*15 is expressed serologically as B62, DQB1*03 is expressed serologically as a DQ8.					

Fig. 3. RCe012-A (RC-8) was analysed by Giesma staining of 20 metaphase spreads at passage 23 and showed a normal 46XX female karyotype in 17 of 20 spreads. Abnormalities in the three remaining cells are believed to be results of harvesting artefacts. An independent assessment of karyology on the same cell line at passage 54 indicated normal 46XX karyology all 20 cells examined.